Original article:

Nasopharyngeal SARS-CoV-2 Viral Load Response among COVID-19 Patients Receiving Favipiravir

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Summary:

We retrospectively studied nasopharyngeal SARS-CoV-2 viral load in the COVID-19 patients who were hospitalized between 13 January and 1 April 2020. Quantitative real-time reverse transcription-PCR were conducted with primers and probes targeting the *ORF1ab* and *N* genes. All patients were classified as Group 1: Received favipiravir + chloroquine or hydroxychloroquine + lopinavir/ritonavir or darunavir/ritonavir for 5-10 days, Group 2: Received chloroquine or hydroxychloroquine + lopinavir/ritonavir or darunavir/ritonavir for 5-10 days and Group 3: no anti-viral medication. Of 115 patients, 38 (33%), 54 (47%), and 23 (20%) patients were in Group 1, 2, and 3, respectively. Median (IQR) baseline viral loads at days 0 of Group 1, 2, and 3 were 7.2 (6.0-8.1), 6.9 (5.8-7.8), and 6.9 (5.8-7.6) $\log_{10}$ copies/mL, respectively. The reductions of mean viral loads at day 3 from baseline were 2.41, 1.38, and 2.19 $\log_{10}$ copies/mL in the corresponding groups (P <0.05). There were no differences in reductions of mean viral loads from baseline among three groups at days 5 and 10 (P >0.05). By multiple logistic regression analysis, receiving favipiravir was associated with nasopharyngeal viral load reduction at three days (P=0.001). Significant nasopharyngeal SARS-CoV-2 viral load reduction was achieved in the COVID-19 patients who received favipiravir-containing regimen.

Introduction

The first confirmed coronavirus disease 2019 (COVID-19) case outside mainland China occurred in Thailand, in January 2020 (1). This contagious disease is caused by novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a newly emergent coronavirus (2). The clinical syndrome appears to have a wide range of disease severity; ie, asymptomatic, mild, non-severe pneumonia, severe pneumonia, and critical illness (3). According to the World Health Organization (WHO) clinical management of COVID-19, patients with moderate to severe COVID-19 should be monitored closely, as pulmonary disease can rapidly progress (3). Providing immediate supplemental oxygen to patients with emergency signs, such as severe respiratory distress, is advised. Remdesivir is recommended as an anti-viral replicating agent as well as glucocorticoids as an immunomodulator to calm the cytokine storm in patients with severe COVID-19 by the infectious disease society of America (4). Nevertheless, Thai clinical practice guideline of COVID-19 recommends a number of specific antiviral combination treatment based on disease severity (5). Briefly, combination of favipiravir with chloroquine or hydroxychloroquine and lopinavir/ritonavir or darunavir/ritonavir is recommended for the patient with pneumonia and oxygenation at ambient air of less than 95%. A combination of chloroquine or hydroxychloroquine and lopinavir/ritonavir or darunavir/ritonavir is recommended for any symptomatic patients with high risk, chronic lung disease, chronic kidney disease, cardiovascular disease, for examples. However, the virologic treatment outcome of such regimens is relatively limited. We therefore retrospectively studied nasopharyngeal SARS-CoV-2 viral load response in the COVID-19 patients who did and did not receive favipiravir-containing regimen as well as mild COVID-19 patients who received usual supportive care but did not receive any specific antiviral treatment.

Materials and Methods

This is a retrospective cohort study conducted among patients with real-time polymerase chain reaction (RT-PCR) confirmed COVID-19 who were hospitalized at the Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Thailand between 13 January and 1 April 2020. The inclusion criteria were (1) all patients diagnosed with COVID-19 and (2) documented RT-PCR confirmed COVID-19. The exclusion criteria were (1) patients who had no subsequent RT-PCR after the diagnosis and (2) patients who transferred out before undetectable RT-PCR. All eligible patients were classified according to their antiviral treatment regimens as follows: Group 1: received oral favipiravir 1600 mg twice daily on day 1, followed by 600 mg twice daily + chloroquine or hydroxychloroquine + lopinavir/ritonavir or darunavir/ritonavir, Group 2: received chloroquine or hydroxychloroquine + lopinavir/ ritonavir or darunavir/ ritonavir and Group 3: did not receive anti-viral medication. The duration of antiviral treatment period in group 1 and group 2 was 5-10 days based on patients' clinical response and attending physicians' judgement.

Nasopharynx specimens were collected using synthetic fiber or flocked swabs. Samples were transported in a viral transport medium containing anti-fungal and antibiotic supplements were used. Total nucleic acid or viral RNA was extracted from the specimens and tested with conventional nested RT-PCR for coronavirus family of the first two novel coronavirus cases in Thailand. Both cases were confirmed as Wuhan human novel coronavirus 2019 by two reference laboratories, included the Thailand National Institute of Health, Ministry of Public Health and Emerging Infectious Disease Health Sciences Center, King Chulalongkorn Memorial Hospital, Thai Red Cross Society by using whole-genome sequencing comparison to the Wuhan reference virus (posted in GenBank, accession number MN908947). Nasopharyngeal SARS-CoV-2 viral loads converted from RT-PCR cycle

threshold (Ct) values with standard curve were compared among three groups. The RT-PCR was conducted with primers and probes targeting the *ORF1ab* and *N* genes. Ct values plotted against log10 (concentration) and the coefficient of correlation (R^2) were used to evaluate the linearity of the assay. The analytical sensitivity of qRT-PCR assays using RNA reference standards was 10 copies for viral RNA. Good linear relationships were obtained between target copy numbers and Ct values (R^2=0.999). The lower respiratory tract specimens were collected in the endotracheal intubated patients. The institute's protocol appointed to obtain nasopharyngeal swab samples tested for SARS-CoV-2 RT-PCR assay every other day since admission, until two consecutive negative results at least 24 hours apart were achieved.

The COVID-19 severity was evaluated at the time of discharge and was classified based on the WHO clinical management of COVID-19 (3): mild (the clinical symptoms were mild, and there was no sign of pneumonia on imaging), moderate (fever and respiratory symptoms with radiological findings of pneumonia, but without features of severe pneumonia), severe (respiratory rate ≥30 breaths/minute, oxygen saturation ≤93%, PaO_2/FiO_2 ratio <300, and/or lung infiltrates >50% of the lung field within 24-48 hours), and critical (respiratory failure, shock, and/or multiple organ failure). Asymptomatic infection was defined as patients had no symptoms or signs throughout the course of the disease. The study protocol was reviewed and approved by the ethic committee of Bamrasnaradura infectious diseases institute (S005h/63). Waiver of informed consent when using medical records or other secondary data was considered and approved.

Descriptive parameters were described as mean±standard deviation (SD), median (IQR 25^{th}-75^{th}) and frequencies (%) as appropriate. One-way ANOVA was used to compare means of parameters among three groups. All possible risk factors associated with viral load reduction compared to day 0 were evaluated with a linear regression model. *P* value <0.05

was considered statistically significant. Variables with P <0.05 on univariate analysis were included in the multiple logistic regression model. All statistical analyses were performed using IBM SPSS Statistics Subscription Trial (IBM Corp., Armonk, NY, USA). A $P < 0.05$ was considered to indicate statistical significance.

Results

A total of 141 patients had been hospitalized during the study period. Of all, 115 patients were eligible for further analysis. Sixty-eight (59%) patients were male. Of all, 64 (55%), 26 (23%), 24 (21%), and 1 (1%) patients were diagnosed with mild, moderate, severe, and asymptomatic COVID-19, respectively. Of all, 33 (29%) had chronic co-morbid diseases. Mean±SD length of hospitalization was 14±10 days. Mean±SD time of RT-PCR conversion from detectable to undetectable values was 13±7 days. Table 1 shows baseline characteristics among three study groups. Thirty-eight (33%), 54 (47%), and 23 (20%) patients were in group 1, group 2, and group 3, respectively. Medians (IQR) baseline viral loads of group 1, group 2, and group 3 patients were 7.2 (6.0-8.1), 6.9 (5.8-7.8), and 6.9 (5.8-7.6) $\log_{10}$ copies/mL, respectively. Figure 1 compared mean±SD reductions of nasopharyngeal viral loads at days 3, days 5 and days 10 after treatment initiation to day 0 among the three groups. Mean nasopharyngeal viral load reductions at three days after treatment from baseline were 2.41, 1.38, and 2.19 $\log_{10}$ copies/mL in the corresponding groups (P=0.002). There was no significant reduction of mean viral load among three groups at days 5 and days 10 after treatment (P >0.05). Table 2 shows univariate and multivariate analysis of possible risk factors of SARS-CoV-2 viral load reduction from baseline at days 3. By multiple logistic regression analysis, receiving favipiravir (P=0.001) and male sex (P=0.044) were associated with high nasopharyngeal viral load reduction at three days after treatment.

Discussion

To date, the clinical benefit data of anti-viral replicating agents for COVID-19 remains limited. Our data showed that nasopharyngeal SARS-CoV-2 viral loads at three days after treatment were significantly reduced in the COVID-19 patients who received favipiravir-containing regimen. In the stage of established pulmonary disease, SARS-CoV-2 multiplication and localized inflammation in the lung parenchyma is crucial (6). During this period, patients develop symptoms of cough, chest discomfort, fever and possibly hypoxia; including chest roentgenogram and/or computerized tomography reveals infiltrates (6). Medical treatment at this stage would mainly consist of respiratory support and potential anti-viral medication (6). Therefore, pharmacotherapeutic agents targeted against the virus replication process are the greatest promise when applied early in the course of the illness. In the patients with significant hypoxemia, the concomitant use of corticosteroids is considered to alleviate exaggerated host inflammatory response (4). The median time to viral clearance in a previous study was 11 days after onset of symptoms. However, the estimated median time of PCR conversion was significantly longer in more severe patients than less severe COVID-19 patients (7). Persistent symptom and pulmonary complication could be partially explained by uncontrolled viral replication (7). As recommended by the national guidelines, the patients who received favipiravir were more severe. They were found to have higher baseline viral load than those who had did not receive favipiravir and therefore could underestimate the virologic efficacy of favipiravir on day 5 and 10.

Favipiravir inhibits *in vitro* replication of wide range of influenza viruses and many other RNA viruses, such as arenaviruses, bunyaviruses, and flaviviruses (8-10). Favipiravir inhibits various types of influenza viruses including seasonal strains and is also active against drug-resistant strains of the virus, including M2 proton channel and neuraminidase inhibitors (9). A recent preliminary clinical result provided useful information examined the effects of favipiravir versus lopinavir/ritonavir in combination with inhale interferon (11). The patients

who received favipiravir showed significant improvement in chest imaging compared with those who received lopinavir/ritonavir with an improvement rate of 91% versus 62% (11). In addition, a shorter viral clearance time was found for favipiravir group, i.e. 4 days versus 11 days. Therefore, favipiravir showed better therapeutic responses on COVID-19 in terms of disease progression and viral clearance. Another recent prospective, randomized, multicenter study of favipiravir for the treatment of asymptomatic and mild COVID-19 in Japan showed that favipiravir did not significantly improve viral clearance by day 6 between early at day 1 and late at day 6 (66.7% vs. 56.1%) of treatment initiation. Nonetheless, favipiravir was associated with numerical reduction in time to defervescence, and a significant improvement in fever was observed the day after starting therapy (12). On the other hand, favipiravir showed no apparent antiviral effect against the SARS-CoV-2 virus in vitro at concentrations under 100 μM by Choy K and colleagues (13). Considering SARS-CoV-2, recent study showed that favipiravir has *in vitro* antiviral activity with the EC_{50} of 61.88 μM which was higher than chloroquine and remdesivir (14). To date, there are a number of ongoing trials all over the world to assess the efficacy of favipiravir in the management of COVID-19 (8). As a consequence, further larger trial is needed to support our promising virological outcome.

A number of limitations need to be addressed. First, this study design is not randomized controlled fashion. According to the national clinical practice guideline, patients with severe COVID-19 are recommended to receive favipiravir-containing regimen. Thus, the benefit of favipiravir alone or in combination with other potential drugs is needed. Second, higher SARS-CoV-2 viral load reductions were achieved in the patients received favipiravir-containing regimen at all three time points, but the significant finding was found only at day 3. This finding would be explained by the small sample size. Third, nasopharyngeal swab by trained personnel has been the standard specimens in our setting.

The personnel designated for specimen collection at the study center has been well trained to ensure the consistency of specimen quality. Ultimately, lower respiratory tract specimens were collected for 4 patients with severe respiratory distress syndrome who needed to be intubated. A recent systematic review showed that lower respiratory specimen has a higher positive positive rate than upper respiratory specimen in such scenario (15). All respiratory tract specimens at diagnosis and during the follow-up period of these intubated patients were collected from lower respiratory tract. Therefore, SAR-CoV-2 viral load reduction at each timepoint could be comparable.

The current COVID-19 pandemic has spreaded globally. Repurposed antiviral replicating drugs are all accelerated into treatment. This study showed that nasopharyngeal SARS-CoV-2 viral loads at three days after treatment were significantly reduced in the COVID-19 patients who received favipiravir-containing regimen. Further larger randomized trial is needed to support this virological outcome and clinical treatment outcome.

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Conflict of interest

None to declare.

REFERENCES

1. Pongpirul W, Pongpirul K, Ratnarathon A, et al. Journey of a Thai taxi driver and novel coronavirus. N Engl J Med. 2020 Mar 12;382:1067-8.
2. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan China: the mystery and the miracle. J Med Virol 2020:92:401-2.
3. Guidelines for clinical practice, diagnosis, treatment and prevention of healthcare-associated infection in response to patients with COVID-19 infection. https://www.who.int/publications/i/item/clinical-management-of-covid-19. Accessed September 10, 2020.
4. Infectious diseases society of America guidelines on the treatment and management of patients with COVID-19. https://www.idsociety.org/practice-guideline/covid-19-guideline-treatment-and-management/. Accessed September 10, 2020.
5. Guidelines for clinical practice, diagnosis, treatment and prevention of healthcare-associated infection in response to patients with COVID-19 infection (8 April 2020). https://ddc.moph.go.th/viralpneumonia/eng/file/guidelines/g_CPG.pdf. Accessed September 10, 2020.
6. Siddiqi H, Mehra Met. COVID-19 illness in native and immunosuppressed states: A clinical–therapeutic staging proposal. J Heart Lung Transpl 2020;39: 405–407.
7. Chen J, Qi T , Liu L, et al. Clinical progression of patients with COVID-19 in Shanghai, China. J Infect 2020;80: e1–e6.
8. Agrawal U, Raju R, Udwadia ZF. Favipiravir: A new and emerging antiviral option in COVID-19. Med J Armed Forces India, https://doi.org/10.1016/j.mjafi.2020.08.004.

9. Sissoko D, Laouenan C, Folkesson E, et al. Experimental treatment with favipiravir for Ebola virus disease (the JIKI trial): a historically controlled, single-arm proof-of-concept trial in Guinea. PLoS Med. 2016;13:e1001967.
10. Bai CQ, Mu JS, Kargbo D, et al. Clinical and virological characteristics of ebola virus disease patients treated with favipiravir (T-705)-sierra leone. Clin Infect Dis. 2014;63:1288–94.
11. Qingxian Cai, Yang M, Liu D, et al. Experimental treatment with favipiravir for COVID-19: an open-label control study. Engineering 2020; Mar 18. doi: 10.1016/j.eng.2020.03.007. Online ahead of print.
12. Doi Y, Hibino M, Hase R, et al. A prospective, randomized, open-label trial of early versus late favipiravir in hospitalized patients with COVID-19. Antimicrob Agents Chemother. doi:10.1128/AAC.01897-20.
13. Choy K, Wong AY, Kaewpreedee P, et al. Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. Antiviral Res 2020,178:104786.
14. Wang M, Cao R, Zhang L, et al. Experimental treatment with favipiravir for COVID-19: an open-label control study. Cell Res 2020;30:269–71.
15. Bwire GM, Majigo MV, Njiro B, et. Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: A systematic review and meta- analysis. J Med Virol 2020; Jul 24;10.1002/jmv.26349.doi: 10.1002/jmv.26349.

Figure 1. Reductions of mean±SD SARS-CoV-2 viral loads from baseline at days 3, 5, and 10

Table 1. Baseline characteristics among three study groups.

Characteristics	**All Cases**	**Group 1 (n=38)**	**Group 2 (n=54)**	**Group 3 (n=23)**	***P* value**
Clinical parameters					
Age, years, mean±SD	40±15	49±17	38±13	32±11	<0.001
Sex, number (%)					0.175
- Male	68 (59%)	27 (71%)	28 (52%)	13 (56%)	
- Female	47 (41%)	11 (29%)	26 (48%)	10 (44%)	
Days of symptoms prior to admission, days, mean±SD	4.2±3.0	4.2±3.2	4.3±3.1	3.9±2.8	0.902
Severity of COVID-19					
- Asymptomatic and mild	65 (56%)	3 (8%)	39 (72%)	23 (100%)	<0.001
- Moderate	26 (23%)	20 (53%)	6 (11%)	0	<0.001
- Severe and critical	24 (21%)	15 (39%)	9 (17%)	0	0.002
Smoking, number (%)					0.537
- Never	79 (69%)	30 (79%)	33 (61%)	16 (70%)	
- Ever	14 (12%)	3 (8%)	8 (15%)	3 (13%)	
- Unknown	22 (19%)	5 (13%)	13 (24%)	4 (17%)	
Co-existing conditions, number (%)					
- Any co-existing conditions	33 (29%)	19 (50%)	12 (22%)	2 (9%)	0.001
- Diabetes	10 (9%)	6 (16%)	4 (7%)	0	

- Chronic kidney disease	2 (2%)	0	0	0	
- Chronic pulmonary diseases	3 (3%)	2 (5%)	1 (2%)	0	
- Chronic heart diseases	1 (1%)	0	1 (2%)	0	
- Hypertension	20 (17%)	4 (10%)	11 (20%)	5 (22%)	
- Hyperlipidemia	7 (6%)	3 (8%)	4 (7%)	0	
- Others	0	0	4 (7%)	1 (4%)	
Initial laboratory findings					
White blood cell count, x10^9 /L, mean±SD	6.05±2.49	5.80±1.99	6.19±2.89	6.11±2.32	0.767
Absolute lymphocyte count, x10^9/L, mean±SD	1.75±1.10	1.61±1.27	1.62±0.62	2.24±1.48	0.035
Percentage of lymphocyte count, %, mean±SD	30±13	27±14	29±11	35±11	0.051
Hemoglobin, x10^9/L, mean±SD	13.5±1.8	13.3±1.9	13.8±1.8	13.1±1.9	0.189
Platelet count, x10^9/L, mean±SD	239±86	216±76	242±81	269±105	0.062
Aspartate aminotransferase (n=64), U/L, mean±SD	32±20	41±23	28±16	19±3	0.008
Treatment Outcomes					

Duration of positive RT-PCR, mean±SD	13±7	13±6	15±8	13±6	0.048
Dead, number (%)	2 (2%)	1 (3%)	1 (2%)	0	0.745
Respiratory failure, number (%)	3 (3%)	2 (5%)	1 (2%)	0	0.408
ARDS, number (%)	4 (3%)	3 (8%)	1 (2%)	0	0.177
Length of hospitalization, days, mean±SD	14±10	19±11	20±9	16±8	0.150

Table 2. Univariate and multivariate analysis of SARS-CoV-2 viral load reduction at day 3 from baseline as the dependent variable

Parameters	**Univariate analysis**		**Multivariate analysis***	
	***P* value**	**Beta**	***P* value**	**Beta**
Receiving favipiravir	0.003	-0.937	0.001	-1.015
Male gender	0.133	-0.488	0.044	-0.618
Hemoglobin	0.106	-0.142		
Diabetes	0.160	-0.865		
Any comorbidity	0.197	-0.502		
Chronic lung disease	0.235	-0.932		
Age	0.253	0.012		
Severe and critical disease severity	0.487	-0.306		
Moderate disease severity	0.496	0.260		
Lymphocyte count	0.757	0.049		
Aspartate aminotransferase	0.903	0.002		
Platelet count	0.962	-0.001		
Mild disease severity	0.947	-0.022		
White blood cell count	0.983	-0.002